



# Serum Prohepcidin and Hepsidin Levels in Patients with Ankylosing Spondylitis: A Prospective Study

## Ankilozan Spondilitli Hastalarda Serum Prohepsidin ve Hepsidin Düzeyleri: Prospektif Çalışma

Ankylosing Spondylitis and Prohepcidin

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### Özet

**Amaç:** Anemi, Ankilozan spondilit (AS) gibi enflamatuar hastalıklarda sık görülen bir komplikasyondur. Son veriler hepsidinin demir homeostaz ve metabolizmasında rol aldığını ve aneminin önemli bir aracısı olduğunu göstermektedir. Bu çalışmanın amacı, AS'li hastalarda ve sağlıklı kontrollerde serum hepsidin ve hepsidinin prohormonu olan prohepsidin düzeylerinin değerlendirilmesidir. **Gereç ve Yöntem:** Bu çalışmaya, AS'li kırk hasta (13 anemi olan ve 27 anemi olmayan) ile 20 sağlıklı katılımcı prospektif olarak alındı. Tam kan sayımı, eritrosit sedimentasyon hızı, serum hepsidin, prohepsidin, demir, total demir bağlama kapasitesi, ferritin, transferrin, ve C-reaktif protein düzeyleri ölçüldü. **Bulgular:** Serum prohepsidin ve hepsidin düzeyleri sağlıklı kontrol ile karşılaştırıldığında, AS'li hastalarda anlamlı olarak yüksek ( $p < 0.005$ ) bulundu. AS'li hastalarda, serum hepsidin ve prohepsidin düzeyleri arasında pozitif korelasyon ( $r = 0.725$ ,  $p < 0.001$ ) arasında saptandı. **Tartışma:** Bizim bilgilerimize göre bu çalışma AS'li hastalarda serum prohepsidin ve hepsidin düzeylerinin araştırıldığı ilk çalışmadır. AS'li hastalarda serum prohepsidin ve hepsidin düzeylerinin hastalığın aktivitesi ile yakından ilişkili olabileceği ve AS ile kronik hastalık anemisinin patogenezinde rol oynayabileceği düşünülmektedir.

### Anahtar Kelimeler

Ankilozan Spondilit; Prohepsidin; Hepsidin; Anemi

### Abstract

**Aim:** Anemia is a common complication in patients with inflammatory diseases such as ankylosing spondylitis. Recent data suggest that hepcidin is a major mediator of anemia with a central role in iron homeostasis and metabolism. The aim of this study was to evaluate the serum levels of hepcidin and its prohormone, prohepcidin, in patients with ankylosing spondylitis in comparison with healthy controls. **Material and Method:** Forty patients with ankylosing spondylitis (13 with anemia and 27 without anemia), 20 healthy adults were prospectively enrolled. Complete blood count, erythrocyte sedimentation rate, serum levels of hepcidin, prohepcidin, iron, total iron binding capacity, ferritin, transferrin, and C-reactive protein were measured. **Results:** Serum levels of prohepcidin and hepcidin were significantly higher in patients with ankylosing spondylitis compared to healthy controls ( $p < 0.005$ ). Positive correlation was determined between the serum hepcidin and prohepcidin levels in patients with ankylosing spondylitis ( $r = 0.725$ ,  $p < 0.001$ ). **Discussion:** To the best of our knowledge, this is the first report of serum levels of prohepcidin and hepcidin in the patients with ankylosing spondylitis. Serum levels of prohepcidin and hepcidin are closely associated with disease activity in patients with ankylosing spondylitis and might play a role in the pathogenesis of anemia of chronic disease associated with ankylosing spondylitis.

### Keywords

Ankylosing Spondylitis; Prohepcidin; Hepsidin; Anemia

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## Introduction

Ankylosing spondylitis (AS) is a frequently occurring chronic inflammatory disease that causes arthritis of the spine, and other large joints. Its pathogenesis is not completely understood [1]. It is a member of the group of the spondyloarthropathies with a strong genetic predisposition.

Although causes of anemia are multifactorial, the most common form of anemia in patients with AS is anemia of chronic disease (ACD) [2]. Shortened erythrocyte lifespan, impaired iron metabolism, and impaired erythropoietin response are suggested to be involved in the pathogenesis of ACD which is also called anemia of inflammation [3]. Systemic and/or local (bone marrow) production of cytokines directly or indirectly influence erythropoiesis [4]. The distinction between healthy adults (HA) and ACD is not clear; the commonly used laboratory tests do not necessarily distinguish these common causes of anemia [5]. Conventional laboratory indices of iron status include serum iron, transferrin, and total iron binding capacity, transferrin saturation and ferritin. Although each of these measurements has merit, no single determination gives a reliable index of iron status [6].

Hepcidin, a recently discovered anti-microbial and cysteine-rich cationic peptide, decreases intestinal iron absorption, in addition, inhibits the release of iron from iron storage sites located in macrophages, hepatocytes and enterocytes [7]. It is proposed that hepcidin may be playing a key role in the ACD pathogenesis, due to its effect on iron metabolism, and its close relation with cytokines/inflammation [8,9]. Levels of hepcidin and its prohormone, prohepcidin were found to be increased 100 times during inflammation, which resulted in decrease in iron absorption and retention of iron in macrophages, decrease in serum iron and eventually causing the ACD [9]. The aim of this study was to investigate the role and significance of hepcidin and prohepcidin on the development of ACD which is frequently seen in patients with AS and the possible utilization of serum prohepcidin and hepcidin levels in the differential diagnosis of ACD [9, 10].

## Material and Method

Forty patients with AS (13 with anemia and 27 without anemia) (25 male and 15 female; mean age, 39±10) and 20 HA as a control group (12 male and 8 female; mean age, 31±10) were prospectively enrolled to the study.

Study Design: a prospective study of patients with AS and with healthy adults. The study has been approved by an institutional review board (Ethical Board of Selcuk University Faculty of Medicine) and subjects have given informed consent. All study was carried out in accordance with the World Medical Association Declaration of Helsinki. AS was defined according to the American College of Rheumatology criteria of 1987 (Morning stiffness, arthritis of 3 or more joint areas, arthritis of hand joints, symmetric arthritis, rheumatoid nodules, serum rheumatoid factor, radiographic changes). Detailed histories of all participants were obtained and their systemic and rheumatologic examinations were carried out. Patients with other concomitant hematological diseases like sickle-cell anemia and thalassemia, and heart, lung, kidney and liver diseases and acute or chronic infection, malignancy and current pregnancy or delivery within

6 months were excluded. Participants receiving blood transfusion, erythropoietin and iron treatment and with recent history of bleeding were also excluded.

Complete blood count, erythrocyte sedimentation rate (ESR), serum levels of hepcidin, prohepcidin, iron, total iron binding capacity (TIBC), ferritin, transferrin and C-reactive protein (CRP) were measured. The normal ranges of values were 50 to 170 µg/dl for serum iron, 120-420 µg/dl for TIBC, 192 to 282 mg/dl for serum transferrin, and 15 to 150 ng/ml for serum ferritin. The blood samples were divided into two tubes (a tube with an anticoagulant -EDTA and without an anticoagulant) in the morning at the end of 12-14 hours of fasting. The analysis of serum prohepcidin (Cat No:12K069-3, DRG, Germany) and hepcidin (Cat No:39K119, DRG, Germany) were carried out at room temperature with ELISA kit by using Rayto RT 2100 C microplate reader (Rayto Electronics, China). The levels of serum transferrin were measured using Beckman Coulter test kits (lot no: T911130), iron, TIBC levels with thermo brand test kits (lot no: V36305) in Synchron LX-20 auto analyzer, the CRP levels with Siemens branded test kits (lot no:167504A) in Dade Behring auto-analyzer. Ferritine levels were studied in E170 auto-analyzer using Roche test kits (Cat No: 03737551). Complete blood count was measured with original kits (lot no: A0115) in Cell Dyn 4000 analyzer device. ESR levels were analyzed through Alifax device.

Definition of anemia: Anemia was defined by a hemoglobin (Hb) concentration <13.0 g/dl in males and <12.0 g/dl in females [10]. According to the World Health Organization; mild anemia corresponds to a Hb ≥9.5 g/dl, moderate anemia to a Hb ≥8 g/dl but <9.5 g/dL, and severe anemia to a Hb <8.0 g/dl. The diagnosis of ACD required the presence of reduced transferrin saturation (<16%), normal/reduced serum transferrin with normal/high serum ferritin (>100 ng/mL) [11].

Patients were not eligible for the study if other conditions which could cause anemia or interfere with erythropoiesis were present (malignancy, previous chemotherapy or radiotherapy, connective tissue diseases, infections, other inflammatory diseases, other spondylarthropathies like Psoriasis, Crohn's disease and ulcerative colitis).

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 18.0, Chicago, IL, USA). The distribution of the variables was analyzed with the Kolmogorov-Smirnov test. The results were expressed as mean ± SD. The comparisons between two groups were assessed using Mann-Whitney U test. Parametrics were performed using the independent-samples T test. Correlation analysis was performed using Pearson correlation tests. P values of <0.05 were considered to indicate statistical significance.

## Results

Baseline characteristics of patients with AS and healthy controls were shown in Table 1. Serum levels of prohepcidin in patients with AS (185.15±44.93) were significantly higher than healthy controls (123.60±18.63, p<0.05). A positive correlation was demonstrated between serum levels of prohepcidin and CRP in patients with AS (p<0.005).

Serum levels of hepcidin in patients with AS (73.77±15.36) were significantly higher than healthy controls (45.82±10.71, p<0.05).

Table 1. Baseline characteristics of patients with ankylosing spondylitis, patients with iron deficiency anemia and healthy controls. Results were presented as mean±SD.

Characteristics	AS Group	Control Group	P Values
Age, yr	39±10	31±10	0.100
Hemoglobin, gr/dl	13.34±1.71	14.11±1.04	0.236
MCV, fl	83.36±6.61	88.96±3.35	0.055
ESR, mm/h	28.37±16.93	10.75±7.8	0.001
Prohepcidin, mg/dl	185.15±44.93	123.60±18.63	0.050
Hepcidin, mg/dl	73.77±15.36	45.82±10.71	0.01
Fe, µg/dl	36.53±21.65	89.65±15.49	0.001
TIBC, µg/dl	227.93±62.16	315.80±21.08	0.001
Ferritin, ng/ml	67.13±64.07	69.0±31.78	0.608
Transferrin, mg/dl	143.79±58.39	205±26.55	0.050
CRP, mg/dl	19.62±14.03	2.6±0.14	0.001

AS = Ankylosing spondylitis; IDA = Iron deficiency anemia; MCV = Mean corpuscular volume; ESR = Erythrocyte sedimentation rate; TIBC = Total iron binding capacity; CRP = C-reactive protein

Hemoglobin levels (13.34±1.71) were similar between AS group and healthy controls (14.11±1.04). Serum levels of iron in AS (36.53±21.65) were significantly lower than healthy controls (89.65±15.49,  $p<0.05$ ). Serum levels of TIBC in patients with AS (227.93±62.16) were significantly lower than healthy controls (315.80±21.08,  $p<0.05$ ).

No significant difference in serum levels of ferritin was found between patients with AS (67.13±64.07) and healthy controls (69.0±31.78,  $p>0.05$ ). Serum levels of transferrin in AS group (143.79±58.39) were significantly lower than healthy controls (205±26.55,  $p<0.05$ ).

ESRs in patients with AS (28.37±16.93) were significantly higher than healthy controls (10.75±7.8,  $p<0.05$ ). Serum levels of CRP in patients with AS (19.62±14.03) were significantly higher than healthy controls (2.6±0.14,  $p<0.05$ ).

## Discussion

Our data mainly suggest that serum levels of hepcidin and prohepcidin are significantly higher in patients with ankylosing spondylitis compared with healthy controls. To our knowledge, this is the first reported study to measure serum levels of hepcidin and prohepcidin in patients with AS. Hepcidin production was shown to be increased in vivo and in vitro experimental and clinical inflammation models [9,12]. It is exclusively produced in the liver and circulates in plasma, consistent with its postulated role as a hormone involved in iron homeostasis [13,14]. Further, hepcidin mRNA expression is increased in response to inflammatory stimuli such as lipopolysaccharide and infection [15]. Although it has not yet been shown to interact with proteins of iron transport, its apparent activity suggests that hepcidin directly regulates the iron transport machinery [16]. Nemeth et al indicated that urinary hepcidin excretion is increased when compared to the control group in acute inflammation [9]. Malyzka et al and Dallalio et al reported increased prohepcidin levels in the chronic hemodialysis patients [17,18]. Demirag et al showed that hepcidin levels were positively correlated with disease activity and negatively correlated with hemoglobin values in rheumatoid arthritis [19]. Hepcidin levels in patients with active ankylosing spondylitis increased when compared to

patients with inactive ankylosing spondylitis [11]. In our study, serum prohepcidin and hepcidin levels in AS group were significantly higher than healthy controls. It was reported that hepcidin production increases in iron load [7,9], and decreases in rats fed with low iron [15]. In clinical studies levels of urinary hepcidin [9] and serum prohepcidin [11] were shown to be high in ACD group in comparison to HC group. In our study, levels of prohepcidin and hepcidin were higher in the ACD group than healthy control group.

Serum levels of transferrin were reported to be more useful than serum levels of iron and total iron binding capacity in measuring the body iron status. Kahgo et al, in their study, indicated that serum levels of soluble transferrin receptor reflected the cellular iron shortage and could be used in differential diagnosis of ACD [20]. In our study, serum levels of transferrin in AS group were significantly lower than healthy controls. Serum ferritin level is the most frequently used laboratory parameter to distinguish between ACD and healthy group [21,22]. Serum levels of ferritin increases as acute phase reactant in AS. Hepcidin is known to be closely associated and positively correlated with ferritin [9,18] but there are also reports of correlation between prohepcidin and ferritin levels [17,23-25]. A positive correlation was demonstrated between serum levels of prohepcidin and ferritin in chronic renal failure [17]. Furthermore, Nagashima et al reported that serum levels of prohepcidin negatively correlated with levels of ferritin in patients with viral hepatitis C, while this correlation was positive in patients with viral hepatitis B and healthy controls [23]. On the other hand, in other studies serum levels of prohepcidin were reported as unrelated with ferritin or other iron parameters [24,25]. In our study, no significant difference in serum levels of ferritin was found between healthy controls and patients with AS. Literature data point to raised CRP concentration as a marker of systemic inflammation in AS patients [26]. In our study, serum levels of CRP were significantly higher in AS group than healthy controls. Positive correlation was determined between serum levels of CRP and prohepcidin. Erythropoiesis is highly dependent upon iron availability, and the most common nutritional cause of anemia is iron deficiency [4]. Normally, most iron used for erythropoiesis is recovered from the degradation of red blood cells by reticuloendothelial macrophages. When this recycling process is inefficient or macrophage iron release is inhibited, serum transferrin saturation falls and erythropoiesis is impaired [2]. Infection, malignancy and chronic inflammation all may result in inefficient iron release from macrophage and subnormal intestinal iron absorption, contributing to the anemia of chronic disease. These alterations have the effect of limiting the availability of iron to red blood cell precursors, even though total body iron stores may be adequate early in the course of the anemia. Some investigators have hypothesized that elevated levels of cytokines induce changes in normal transfer of iron between macrophages and developing red blood cells, and some cytokines have been shown to alter the expression of macrophage transferrin receptor and ferritin, but there is currently no direct evidence that any particular cytokine inhibits cellular iron egress [27]. Classically, ACD is associated with low serum iron and TIBC, and high or normal serum ferritin levels [2]. In the present AS patients, serum iron status was consistent with this classical

data. ACD may not only be normochromic-normocytic it may also have hypochromic-microcytic or normocytic features [28]. Vreugdenhil et al have shown that the anemia was normochromic-normocytic in 60% and hypochromicnormocytic in 30% of those with ACD [28]. These data suggest that hepcidin is an important pathogenetic marker in pathobiology of anemia in AS patients without iron deficiency. In our study, in addition to hepcidin, we found that the serum iron level was a significant predictor for Hb level in all AS patients.

This study has some limitations. In inflammatory diseases, can coexist with ACD due to poor intake and/or absorption and increased loss of iron, and so, to differentiate between ACD and iron deficiency anemia may be difficult [3]. Thus, our failure to use more pertinent indicators such as transferrin receptor to distinguish between ACD and iron deficiency anemia may be one of the limitations of the present study. The hemochromatosis gene is an upstream regulator of hepcidin, and it could influence the prohepcidin levels in some individuals, so not to determine the hemochromatosis gene mutation status may be second limitation of this study.

### Conclusions

Serum levels of prohepcidin and hepcidin are closely associated with disease activity in patients with AS and might play a role in the pathogenesis of ACD associated with AS. To the best of our knowledge, this is the first report of serum prohepcidin and hepcidin levels in the patients with AS.

### Competing interests

The author(s) declare that they have no competing interests.

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